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DSTO

Bioaerosols: A Survey

R. Ian Tilley

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DSTO-GD-0163

ABSTRACT

Biological warfare agent aerosols must be detected against a background of native bioaerosols. The latter are described together with their sources, factors affecting their abundance and their sampling and characterisation. Australian bioaerosol studies to date are described and bioaerosol monitoring work needed to address deficiencies in the data on the bioaerosol content of air at Australian locations identified.

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Executive Summary

A modest program in defence against biological warfare agents has recently commenced within DSTO. The drivers for this work include the realisation that biological agents can be relatively easily and clandestinely acquired by rogue nations or non-government groups and that their effects may be devastating to unprotected and unprepared personnel. It is considered important that DSTO have the ability to provide timely and accurate scientific and technical advice to the ADF should they be required to operate in an environment contaminated with biological material. The latter is most likely to be in the form of a bioaerosol.

An important aspect of the program concerns detection of "foreign" bioaerosols against a background of naturally occurring bioaerosols. The first part of this report examines the nature of naturally occurring bioaerosols, their sources, factors affecting their presence in the air (e.g., weather conditions, time of day or year, human activity) and techniques for their monitoring and identification.

The second part of the report identifies known Australian activity in bioaerosol monitoring and characterisation. It was found that such activity was mostly confined to the monitoring of pollens and fungi which may cause allergenic reactions (e.g., hay fever and asthma). No routine monitoring of the air for bioaerosols is conducted at present in Australia. Consequently it is considered prudent that preliminary measurements be made of the background bioaerosol levels at some militarily significant sites in northern Australia.

The outcome of such work will include a broadening of DSTO's expertise in this area which will enable appropriate advice to be given to the ADF on operating in an environment contaminated with biological agents. It will also help to establish DSTO's credentials as a legitimate player in BW defence in the international defence community and facilitate access to valuable information held by other countries.

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1. Introduction

Recent revelations[1] on the extent of Iraq's biological weapons program have focussed international attention on biological warfare (BW). The relative ease with which Iraq's clandestine program was established has given rise to fears that other determined states may see BW as an attractive option in future conflicts. At the moment the perceived risk to Australia of direct use of biological weapons against it or its defence personnel is minimal.[2] However the possibility that in the future the ADF may be required to operate in a biologically contaminated environment cannot be discounted. For this reason and with Australia's commitment to the abolition of all weapons of mass destruction (nuclear, chemical and biological) a moderate program of research into defence against biological weapons is maintained.[2]

A BW agent is a microorganism or toxin which causes disease in humans, animals, or plants, or which causes the deterioration of material. Classes of BW agents harmful to humans (examples of each class in parentheses) include bacteria (anthrax, plague), rickettsia (Q fever, typhus), viruses (small pox, Ebola) and toxins (botulinum, ricin). BW agents can be disseminated through water and food or through the use of vectors such as fleas or ticks, but a more effective method is through inhalation of aerosolised particles.[3]

Current protective masks and clothing provide complete protection against biological aerosols (bioaerosols) but the wearing of these items degrades human performance. Hence it is considered essential to have an effective BW warning and detection capability so that (i) personnel can take appropriate protective measures and (ii) personnel can relax protective posture for the maximum possible time. Monitoring equipment could be used to detect and possibly characterise biological aerosols, however efficient use of such equipment will rely on the ability to distinguish between the natural or background bioaerosol content of the air and BW agent aerosols.
This report discusses the nature of bioaerosols, instrumentation for their detection and characterisation, existing Australian data, Australian organisations working in the bioaerosol area and recent developments in BW agent detection equipment. Suggestions are also made regarding the future direction of DSTO's research in BW detection.

2. The Nature of Biological Aerosols

2.1 General

Atmospheric aerosols may range in diameter from 0.002 to 100 μm.[4] This report confines itself to respirable particles which are defined as airborne particles capable of reaching the alveolar or pulmonary region of the human lung.[4] Studies made in the 1950's found that particles greater than 4-5 μm diameter were completely screened out

by the nose and did not reach the lungs[5,6]. It was also found that (i) the screening effect decreases with particle size to zero for 1 μm particles, (ii) that depth of penetration into the respiratory tract increases with decreasing size, (iii) that retention in the lung is complete for particles greater than 1μm which get past the nose, and (iv) that retention in the lung decreases in going from 1.0 to 0.25 μm particles[6]. From the above it appears that particles between 0.25 and 5 μm are of most interest in terms of their ability to reach, and be retained in, the human lung.

Particles or colonies of organisms greater than 5μm in the largest dimension that lodge in the nose and back of the throat will still be capable of causing infection and will be likely to be trapped in the mucus and swallowed. Since the large particles will settle more rapidly than those less than 5μm they are less of a threat.

2.2 Types of Bioaerosols

Airborne biological material may contain bacteria, viruses, fungi, pollen and pollen fragments, bacterial and fungal spores, plant and fungal debris, protozoa and algae. Viruses range in size from 0.015 to 0.45 μm, bacteria from 0.3 to 10 μm, fungal spores from 1.0 to 100 μm and pollen from 3.5 to >200 μm.[7,8] Organisms too small to be retained by the human lung may still lodge there when they are agglomerated or attached to other atmospheric particles such as dust. Although pollen particles are generally too large to pass the nasal channel, they can fragment into smaller particles which can reach the inner lung.[8]

2.3 Sources of Bioaerosols

2.3.1 Natural Sources

Natural sources of bioaerosols include animals (mammals, birds, insects), man, soil, plants, fungi and other decomposition products in soil and water bodies. This decaying matter and the excrement of animals are sources of bacteria which can become airborne under favourable conditions. Animals and man shed skin flakes and hair which may be sources of bacteria, viruses and fungi. People discharge bacteria and sometimes viruses into the air when they talk, cough and sneeze, although the contribution from these sources to the overall bioaerosol content is considered to be small.[9]

Soil contains bacteria and fungi which may become airborne as individual particles, aggregates or particles formed by their attachment to dust nuclei. These may be raised by wind or other disturbance, e.g., animal or human movement. Rain splash can also release biological particles into the atmosphere.

Plants, which include trees, grasses, weeds and flowering varieties are sources of pollen, seeds, and leaf and stem detritus. Decaying plant matter is also a source of bacteria and fungi.

Bodies of water including the oceans, lakes, rivers and stagnant pools are sources of biological matter particularly bacteria. The latter may be released into the air through the breaking of waves, wind spray and foam, and the bursting of bubbles at the surface of a body of water.

2.3.2 Sources due to Human Activity

Activities associated with agriculture which can generate bioaerosols include tillage of soil, spraying of wastewater or biological materials, harvesting of crops, milling or other processing operations, and transport and storage of grain and seeds.
Sources of bioaerosols in the livestock industry include poultry farms, piggeries, dairies and abbatoirs. Any activity involving the handling of animals or birds or the processing of animal products is a potential source of bioaerosols.
Industries which are sources of bioaerosols include sewage treatment plants, cooling towers, textile mills, fermentation processes (brewing, microbiological products, dairy products), vehicle movement and building and auto demolition.

3. Factors Affecting Bioaerosol Content of the Air

The amount of biological particles suspended in the air is affected by location, temporal factors and atmospheric variables.

3.1 Location

In general small numbers of biological particles are found in the air over oceans; the particle count increases over the land in rural areas and is greatest over urban areas. The count also decreases with altitude which is mainly a function of gravitational settling of particles. Proximity to a local source, such as a sewage treatment plant or an area of pollinating plant life, will also influence the bioaerosol content of the air.

3.2 Temporal Factors

Studies of airborne bacteria[10], fungal spores[11] and pollen[12] showed concentration changes with time of day. The viable bacterial count at a desert site was lowest at around noon, which suggested that damage by solar radiation was responsible.[10] In work reported by Bhati and Gaur[11] the maximum number of fungal spores was obtained in the forenoon period, followed by afternoon and night. Hurtado and Alson[12] found that the number of airborne *Cecropia* pollen grains was very low in the

pre-dawn period, increased to a maximum at noon, decreased slightly in mid-afternoon, and reached a second maximum at 6 pm.

Seasonal variations are inevitably found in counts of airborne biological particles. Commonly, counts are highest in the warmer months (spring, summer and autumn) and lowest in winter. Pollinating plants are active during the warmer months when conditions more favourable for the production and survival of bacteria and fungi occur. Moreover, atmospheric convective effects, which assist in the suspension of aerosols, are normally more frequent in the warmer months.

Variations in bioaerosol content and character for different years have been reported by some workers.[12-15] Bacterial and fungal concentrations differed significantly in 2 years of observations in a Washington, D. C. suburban area.[13] Airborne pollen counts have also been shown to differ between years.[12,14,15] This results from changes in the time of onset of pollen release due to variations in climatic conditions in different years. For example, after an unusually short warm winter, many trees bloom early in the season but an unusually long winter will postpone the time of pollination.[14]

3.3 Atmospheric Variables

3.3.1 Air Movement

Movement of bioaerosols in the atmosphere is controlled by the movement of air, so wind speed, direction and atmospheric stability are important in determining the local concentration of bioaerosols.[16] Increased wind speed leads to increased microbial counts in the air.[13,17] Other biological material and dust will also be lifted into the air in increasing amounts and carried further by wind of increasing speed.[15] Strong winds dilute local pollution, but bring other particles in from distant areas.[18] If wind blows from the direction of a known source of biological matter an increased bioaerosol count would be expected. In still conditions, convection currents in the air will influence the biological content of the latter, as has been demonstrated by Soldevilla et al.[19] Long-distance transport of bioaerosols has been observed[9] whereby air masses containing entrained biological particles can travel intercontinental distances before deposition.

3.3.2 Temperature and Solar Radiation

In general, increased temperature is associated with elevated counts in air of microorganisms and fungal spores.[13,15,17,18,20] However some studies have found no correlation between bioaerosol counts and temperature.[13,21] This lack of correlation may be caused by temperature effects being masked by those from the many other variables affecting the counts. It should also be recognised that extremes of temperature will slow the growth of microorganisms or may reduce their survival in the atmosphere. One effect of temperature is to induce air movement ranging from convection currents through to large air circulations, such as sea breezes and mountain

(valley) winds. For a detailed discussion of these effects see Kim.[16] Associations have been found between bacterial size and temperature.[21] Higher temperatures favoured larger particle sizes.

Lighthart and Shaffer have proposed[10] that solar heating of the surface of soil, with resultant turbulent diffusion in soil and plant surface microlayers, could cause release of particles with adherent bacteria to be carried aloft by convective updrafts. Drying may also produce brittle plant epidermal elements with adhering bacteria which may be swept up by the same process. It has been shown[19] that when there is an increase in ambient temperature, and in hours of maximum sunlight, certain species release pollen grains by the explosive opening of the anther.

Temperature and solar radiation also affect the survival or viability in air of microorganisms through evaporation of liquid droplets containing microbes, and through direct damage by the UV content of solar radiation. Evaporation of droplets may expose microbes to damaging atmospheric conditions by removal of the protective liquid sheath. It has also been observed,[10] at a high desert site in the United States, that solar radiation damage to bacteria was size discriminatory. There was a greater effect on smaller size categories at midday and a relatively lesser effect in the morning and evening. The characteristics of this site may approximate conditions found in some parts of northern Australia and consequently are of some interest.

3.3.3 Moisture

Rain showers temporarily clear the air of airborne particles, including those of biological origin.[11,17] This process is called wet scavenging.[16] Increased rainfall and rain in the days immediately prior to sampling have been shown to be associated with increased fungal spore counts.[15,18] However one study found that mean pollen levels were reduced during periods of increased rainfall.[22] This is most likely due to the wet scavenging effect of rainfall noted above.

Cox has discussed in detail the effect of relative humidity (RH) on the stability of biological particles.[23] Different biological particles are most stable at different RH values. Dessication can lead to loss of viability, the former being RH dependent. Particles with robust outer coats (e.g., fungal spores) are least affected by RH. Bovallius et al[17] found that high RH lowered bacterial counts. Lee and coworkers[21] found a negative correlation between bacterial size and RH which suggested that high humidity may favour the survival of small bacterial particles in air. In the same study the number of viable bacteria was only weakly correlated with RH. High RH has also been found to be associated with increased counts of a particular fungal basidiospore closely resembling the genus *Ganoderma*.[18] Jones and Cookson[13] found no relationship between RH and either microbial concentration or particle size distribution. This may again be due to the difficulty of isolating the effect of a single variable from all other influential variables.

3.3.4 Atmospheric Constituents

It might be expected that bioaerosol concentration would be related to the total suspended particulate matter (TSP) in the air because small biological particles are often carried into the air on rafts of plant matter, dust and other inorganic matter. A study in Mexico City in 1991[24] found a significant positive correlation between the protein associated with airborne particles and the total airborne particle count. It was suggested that suspended road dust, which was prevalent in the sampling area, could be an important source of airborne proteins.

A study in an urban area of Cincinnati[21] found only a weak correlation between the concentration of viable airborne bacteria and TSP. The part explanation given was related to the sharp difference between the size of airborne bacteria and the size of TSP. The larger aerodynamic size of airborne bacteria (average 7.6 μm) compared to the submicron size of TSP (average 0.77 μm) afforded little opportunity for bacterial collection on particle surfaces. The Cincinnati study did reveal that when temperature and relative humidity were held constant, significant correlations were revealed between viable bacteria concentration and the concentrations of NO (positive), NO_2 (positive), SO_2 (negative), hydrocarbons (positive) and CO (negative). Bacterial size (mass median diameter) was also negatively correlated with the concentration of CO. The authors of the study were unable to establish a direct cause-effect relationship between the automobile pollutants (NO, NO_2, CO and hydrocarbons) and SO_2 and bacterial morbidity. Any effect of these pollutants on airborne microbes will be reduced markedly, if indeed they are present at all, in non-urban environments such as outback Australia where vehicular traffic is low.

4. Sampling and Characterisation

Detailed descriptions and discussions of sampling and analysis techniques can be found elsewhere[4, 25-30] and only a broad and general review is given here.

4.1 General Considerations

No single sampling method can collect, identify and quantify all of the bioaerosol components in a particular environment.[26] Consequently characterisation of the bioaerosol content of a site is facilitated if some idea of what might be found can be gained. This involves taking an inventory of potential local sources of bioaerosols and obtaining detailed meteorological information for the site. Local water sources, soils and relevant industrial environments can be subjected to microbiological analyses to identify any local bacteria and fungi. Pollinating plants and their pollination seasons can be identified and their pollen characterised. Fungi may be also characterised in this way and their spore production and release mechanisms noted. Meteorological data, including prevailing wind direction, temperature and humidity at different times of

the day and year, can be used to determine the likely exposure of a site to locally-produced bioaerosols.

4.2 Collection of Bioaerosols

4.2.1 General Principles

Bioaerosols are sampled by removing them from the air and retaining them for subsequent analysis. A simple way to do this is to expose a suitable surface to the air and let any biological particles settle on the surface under the influence of gravity. This is the so-called gravitational settling method and typically utilises greased glass slides for collection which can be subsequently viewed under a microscope for identification of biological particles, most often pollen and fungal spores. Vegetative cells such as bacteria and viruses can be trapped in a culture medium such as agar, and colonies grown under suitable conditions.

The method is simple and the equipment inexpensive, but it has limitations in that (i) it is not quantitative given the volume of air sampled is indeterminate, (ii) identification of biological particles under a microscope is time consuming and requires experienced and skilled observers for accurate results, and (iii) different bacteria may require a variety of different growth conditions. The latter usually implies multiple collection slides at a site and a variety of laboratory conditions to promote the growth of differing organisms.

Typical sampling instruments overcome the first shortcoming described above by drawing known volumes of air past the collection device, usually with the aid of a suction pump. Air is drawn through well defined slits, orifices or nozzles and past collecting surfaces or through a liquid where particles are deposited. The removal of particles from the air is achieved in many samplers by abruptly diverting the direction of the incoming air stream. This causes particles having sufficient inertia to continue to travel straight ahead and out of the air stream where they subsequently impact upon the collecting surface or liquid and be retained. Other methods of removing particles of interest from an incoming air stream include electrostatic techniques and filtration.
Some samplers, e.g., the Burkard spore trap, utilise collecting surfaces which can be moved past the air inlet by a drive mechanism. This allows a particle-count/time profile to be obtained. Other devices such as the Andersen sampler[31] allow a particle size distribution to be obtained.

Samplers are subject to factors which reduce their sampling efficiency.[26] For example, inlets and collection surfaces may not respond equally to all types and sizes of bioaerosol particles, overloading may occur if the sampling time is inappropriate or the bioaerosol concentration is high, and the viability of vegetative cells may be compromised by the sampling process. Consequently careful consideration needs to be given to selection of the most appropriate sampler and sampling regime for a given application.

4.2.2 Instrumentation

Widely used samplers include the Rotorod rotating rod impact collector (Sampling Technologies, Inc., St. Paul, MN, USA), the Burkard 7-day recording volumetric spore-trap (Burkard Manufacturing Co. Ltd., UK), the Andersen six-stage viable (microbial) particle sizing sampler (6-STG, Graseby-Andersen Samplers Inc., Atlanta, GA, USA) and the Ace all-glass impinger-30 (AGI-30, Ace Glass, Vineland, NJ, USA).

The Rotorod sampler consists of greased lucite rods connected to a disc which spins at 2400 rpm. Biological particles stick to the rods as they spin through the air. The volume sampled equates to 21.4 L/min. After exposure the rods are mounted, stained and examined under a microscope. The sampler is normally used for collecting pollen and spores and is less suitable for viable microorganisms.

The Burkard sampler draws air through a slit at 10 L/min and particles are trapped on an adhesive tape wound round a drum which rotates once in seven days. A vane on the instrument ensures that the entrance slit faces the prevailing wind. At the end of the sampling period the tape can be removed and examined under a microscope. This instrument is also usually used for pollen and spores and is less suitable for viable microorganisms such as bacteria.

The Andersen six-stage sampler is commonly used to sample viable biological particles and provide a size distribution. The six stages collect particles having the following aerodynamic sizes: 8.5-15 microns (stage 1), 5-10.5 microns (stage 2), 3-6 microns (stage 3), 2-3.5 microns (stage 4), 1-2 microns (stage 5) and <1 micron (stage 6). Air is drawn at 26.8 L/min. through holes in flat circular plates and passed over collecting plates where high inertia particles separate from the airstream and are retained on the collecting plate. The air velocity is increased by making the holes progressively smaller so that large particles are deposited on the first plate followed by successively smaller particles on subsequent plates. After sampling, the collector plates can be removed for analysis. In many cases particles are collected in a nutrient medium which can be subsequently incubated, and colony-forming microorganisms counted and identified.

The All Glass Impinger (AGI-30) is similar to solvent traps or bubblers used to remove chemical vapours from a stream of carrier gas. Air to be sampled is drawn through a narrow glass tube at 10-15 L/min., with an orifice outlet above the surface of a collecting liquid contained in a glass cylinder. The incoming air is accelerated through the orifice and impinges upon the surface of the liquid where entrained particles are released and captured. After sampling, the liquid is removed and any captured particles analysed by appropriate techniques. The AGI-30 is often used to sample air for viable microorganisms.

4.2.3 Analysis and Identification

Biological particles are most commonly counted and identified by microscopic techniques. These include conventional light microscopy, phase contrast microscopy,

dark field illuminated microscopy, polarizing microscopy, interference microscopy, fluorescence microscopy, confocal microscopy, scanning electron microscopy and transmission electron microscopy.[32] Particles can be stained to distinguish biological particles from inorganic particles. Identification is based on physical appearance of particles and reference to authentic samples. Some preparation of collected samples is usually required before they are viewed under a microscope.

Methods applied, particularly to viable microorganisms such as bacteria and viruses, include fluorescence and luminescence spectroscopy, infrared and raman spectroscopy, mass spectroscopy, flow cytometry, and electrochemical methods and sensors.[33]

Biological and biochemical methods used include antibody-based detection of specific microbial antigens, immunofluorescence techniques, enzyme immunoassays and radioimmunoassays, immunoelectroblot techniques, detection of specific microbial nucleic acids and other products, chromogenic and fluorogenic enzyme substrate tests and gas-liquid chromatography.[34]

Pollen particles and spores of fungi and bacteria are relatively easily characterised by direct microscopic examination. Microorganisms such as bacteria usually require cultivation to enable identification. This involves selection of the most appropriate nutrient media and conditions for their propagation. This complicates the process because different bacteria require different conditions for their growth. It is helpful if the bacteria to be sampled in the air are known beforehand. In a military situation this will not usually be the case. Incubation of nutrient samples to grow viable microorganisms for identification can take days. From a military standpoint this is less than ideal. Real time identification would be desirable and stand-off or remote detection and identification would be ideal.

Recent research and development in the defence science community has concentrated on producing a real or near real time detection capability[35]. A proven stand-off detection system has not yet been developed[36]. Most of the proposed techniques are based on specific binding of antibodies to the organism of interest (antibody-antigen binding). Work has concentrated on developing techniques which rapidly detect the binding event. Some techniques employ labelled adducts which cause light to be emitted after binding occurs, and involve chemiluminescent-, fluorescent- or visible light-emitting adducts. Other techniques employ coated surfaces which undergo, or produce, a physical change when binding occurs. These include piezoelectric crystal microbalances, resonant mirrors, surface plasmon resonators, electrical capacitance biosensors and biorefractometers. The physical changes measured by these techniques are respectively: oscillation frequency, surface refractive index, surface reflectivity, electrical impedance and surface refractive index.

Other techniques under development attempt to directly identify biological entities. These include mass spectrometry (MS), capillary electrophoresis and gene probes. The mass spectrometry techniques under development are electrospray ionisation,

pyrolysis MS and matrix-assisted laser desorption ionisation (MALDI). Whole cells, protein extracts and phospholipids have been studied. The capillary electrophoresis and gene probe technologies are designed to recognise characteristic molecular features of biological molecules of interest.

Arguably the most successful current instrument for bioaerosol detection is the Canadian-developed fluorescence aerodynamic particle sizer (FLAPS). This incorporates a commercially available aerodynamic particle sizer combined with a laser-based fluorescent detector which identifies the biological content of an aerosol. The instrument does not identify individual species of microorganisms or pollen for example. However a useful indication of the biological character of a measured bioaerosol is provided. Once the background biological nature of the atmosphere at a site is established any unusual bioaerosols can be detected and further tests can be run to determine the exact nature of these bioaerosols. In a military situation the FLAPS instrument can act as a sentry to warn of possible BW attack, and allow protective measures to be taken until the all-clear is given. The FLAPS could be used to give an initial indication of the biological aerosol content at a given location.

5. Australian Bioaerosols

In Australia the air is not routinely monitored for biological content. Australian organisations with the responsibility for air monitoring such as the Environment Protection Authorities in the States and Territories do not consider airborne biological particles to be air pollutants hence the lack of monitoring activity. The air at various localities is monitored for total suspended particulates, but no steps are taken to distinguish biological particles from the other suspended matter such as dust and smoke.

Bioaerosol studies in Australia have been concerned with aeroallergens (pollen and fungi)[8,18,37,38], which may trigger asthma and hay fever attacks in susceptible persons, and workplace exposures to microorganisms[39,40,41]. The NSW Health Department's Health and Air Research Program (HARP) monitored pollen levels in the Sydney, Hunter and Wollongong areas in 1992 and 1994[37]. The main constituents found in the air were grass and tree pollens and the fungus *Alternaria*. The Botany Department of Melbourne University has monitored pollen levels in Melbourne since 1991 using a Burkard 24-hour volumetric recording sampler[38]. An airborne pollen calendar was produced and it was found that tree pollen accounted for about 62% of the catch, grass pollens 20% and pollen from herbs and weedy plants 9%. Investigators from the University of Queensland and Griffith University monitored the air in Brisbane for atmospheric particulate matter in 1992[18]. Here fungal spores dominated the count in the 2-10 micron range but, even at peak periods, the total bioaerosol count only contributed about 5-10% of the total particulate mass.

WorkSafe Australia sponsored a study on sampling methods for microbial contamination in indoor work environments[39]. Gravity settling plates were compared with the Andersen 6-stage cascade impactor. Reasonable agreement between the two samplers was found for fungi and bacteria. The major fungi genera found were *Cladosporium* and *Penicillium*. Gravity settling plates may be suitable for indoor sampling where air movement is not great. However, in outdoor environments they may not be as useful because increased air movement will prevent smaller particles settling on the plates resulting in counts biased towards larger, heavier particles. Investigators at the Victoria University of Technology are interested in the exposure of individuals to bioaerosols. A bioaerosol chamber has been designed and constructed to allow performance evaluation of bioaerosol sampling devices[40]. A 1992 study in Queensland raw-sugar mills monitored levels of airborne *Thermoactinomyces sacchari* spores using 6-stage cascade impactors (Andersen and May/Research Engineers, London, UK) and Mylar filters[41]. Phase contrast light microscopy at 1000x magnification was used to identify the bacteria spores which can cause bagassosis to develop in exposed individuals. Microscopy was also used in a Canberra study on suspended particulates which included pollen[42].

Other areas where bioaerosol monitoring occurs include hospitals, "sick" buildings, cooling towers (*Legionella*), clean rooms in the pharmaceutical industry, abattoirs and other primary industry workplaces. Organisations and institutions which may measure bioaerosols include tertiary colleges and university departments of botany, microbiology, environmental and biological science, atmospheric science and medicine, Government Health Departments, hospital laboratories, Environment Protection Authorities, CSIRO, WorkSafe Australia and other occupational health and safety bodies, and commercial environment consultants. These measurements are not always reported in the open literature.

6. Discussion

The background bioaerosol content of Australian air has not been well characterised to date. The only significant studies have involved measures of the pollen content of the air at population centres in eastern Australia. Given this absence of data, a survey of bioaerosols in northern Australia is warranted to enable the native or background aerosol to be characterised. This should involve sampling and characterisation of airborne pollens, fungi, bacteria and other proteinaceous material. Particles of interest would be in the range 0.25 to 20μm. Conventional techniques could be applied initially, and the survey done by an organisation or institution with expertise in air monitoring and analysis, and characterisation of airborne biological particles. Access to microscopic and microbiological techniques and facilities would also be required.

Appropriate monitoring sites of defence significance could include sea and air ports and fixed military installations such as communications and logistics bases and command and control centres. A broad indication of the background biological content

of the air could be obtained. DSTO staff would also become familiar with the monitoring techniques and instrumentation.

A second stage could involve operation of the Canadian FLAPS instrument alongside the conventional instruments to gain an understanding of the capabilities of FLAPS. This instrument shows promise as an indicator of the presence of foreign bioaerosols and may have a role to play in the early warning of a BW attack. Its ability to operate in northern Australian conditions could be assessed.

After a site for monitoring had been selected its relevant features would be documented. These would include (i) geographical features such as plains, rivers or streams, grasses, trees and soil type, (ii) local sources of biological particles such as lakes, agriculture, livestock, poultry, decaying plant or animal matter, and industrial facilities, and (iii) other details such as the volume of local traffic (people, vehicles and animals).

Bacteria should be trapped using an Andersen sampler or slit-to-agar sampler; pollen and fungal spores should be sampled with a Burkard spore trap or similar instrument. Pollen and fungi could be counted and classified using microscopy, and bacteria cultured and identified as gram-negative or gram-positive and the genus and physical characteristics determined (e.g., bacillus, cocci, etc.). Meteorological data would need to be recorded and would include air temperature and humidity, wind speed and direction and solar radiation.

7. Conclusions

1. The total native or background bioaerosol present at Australian sites has not been characterised to date.

2. Measurements of the total background bioaerosol at militarily significant sites in Australia should be made to enable meaningful evaluation of BW detection equipment which may be required by the ADF for operations in contaminated environments.

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19. ABSTRACT

Biological warfare agent aerosols must be detected against a background of native bioaerosols. The latter are described together with their sources, factors affecting their abundance and their sampling and characterisation. Australian bioaerosol studies to date are described and bioaerosol monitoring work needed to address deficiencies in the data on the bioaerosol content of air at Australian locations identified.